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THE DETERMINATION OF THE LEVELS OF DINITROPYRENES ON DIESEL EXHAUST PARTICULATE EXHIBITING EXCESS MUTAGENIC ACTIVITY

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MICROMEDIA

A B S T R A C T

High volume samples of diesel exhaust particulate have been collected downstream from two different Deutz 714 series diesels fitted with Dieseler III catalytic converters. The samples were collected on a level underground site with an 800 foot tramming distance to the ore pass. This should have ensured that the diesel particulate and the absorbed PAHs would have been stored and nitrated (converted into the highly mutagenic nitroPAHs) in the catalyst during the long tramming portion of the LHD cycle. In spite of this, only one out of six diesel exhaust extracts was mutagenic in the Ames bioassay using Salmonella tester strain TA-98 + S9. Even the one sample that contained mutagens was only slightly mutagenic, i.e., the exhaust was only as mutagenic as the exhaust from a bare engine. Thus, none of the samples contained significant quantities of the highly mutagenic nitroPAHs.

The diesel exhaust extracts from the samples collected in this study differed from those found in other studies in two ways. First, these extracts were

several times more toxic to the Salmonella bacteria used in the Ames bioassay than any of the previous extracts. Secondly, these extracts contained a DMSO insoluble material whereas all previous extracts dissolved completely in the DMSO used in the Ames bioassay. Neither the structure of the DMSO insoluble material nor the source of the toxicity have been discovered.

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INTRODUCTION

In previous studies (1,2), the soluble organic extract from diesel particulate collected on High Volume filters downstream from heavy duty Load Haul Dump (LHD) diesel engines fitted with Dieseler III catalytic converters in an underground mine was much more mutagenic than the organic extract from LHD diesels fitted with either no or other types of catalytic converters or filters.

The very high mutagenicity of the soluble organic extracts from diesel exhaust particulate collected downstream from diesel engines fitted with the Dieseler III catalytic converters in the underground environment was tentatively attributed to the presence of nitroPAHs that were formed in the catalyst when the polynuclear aromatic hydrocarbons (PAHs) that were on the particulate trapped by the catalyst reacted with the hot nitrogen dioxide in the diesel exhaust. The equations in Figure 1 illustrate how pyrene, one of the most abundant PAHs in diesel exhaust (1), can be converted into the highly mutagenic nitropyrene and subsequently into the even more highly mutagenic dinitropyrene (2).

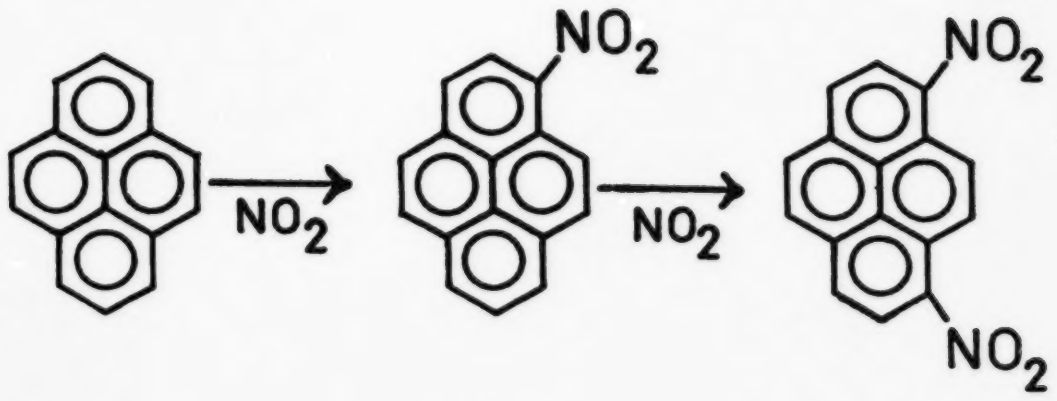

Figure 1. The mononitro-and dinitropyrene (nitroPAHs) formed in the nitration of pyrene and the mutagenicity of the nitropyrenes in the Ames bioassay using Salmonella Typhimurium strain TA-98 without metabolic activation.

The source of the high mutagenicity of the extract from the diesel exhaust collected downstream from diesels fitted with Dieseler III catalysts had been tentatively ascribed to the presence of mononitro and dinitroPAHs in the exhaust. Two different pieces of evidence supported this hypothesis. First, several workers have reported the presence of the highly mutagenic nitroPAHs in exhaust collected from engines burning hydrocarbon fuels (3,4). The second and even stronger piece of evidence, is that the mutagenicity of the diesel exhaust extract is significantly higher when Salmonella Typhimurium bacteria strains that react with mononitro and/or dinitro compounds are used in the Ames bioassays (5,6) whereas significantly lower mutagenicities are found when Salmonella strains that do not react with mononitro and/or dinitro compounds were used in the Ames bioassays. For example, the mutagenicities in Table 1 are much lower when Salmonella strain TA98-NR, which does not react with mononitro compounds, TA98-1,8 DNP_6, which does not react with dinitro compounds, and TA98-NR/BR which will not react with either mononitro or dinitro compounds, are used in the Ames bioassay. This suggests that most, if not all, of the mutagenicity in the diesel exhaust extract from diesels fitted with Dieseler III catalytic converters is due to nitrated compounds, probably the nitroPAHs. The very high mutagenicity of the diesel exhaust extract is consistent with the presence of nitroPAHs because i) both

Table 1. The mutagenicity* of the extract from diesel exhaust samples collected downstream from a Deutz diesel fitted with a Dieseler III catalytic converter as determined in a multistrain Ames bioassay without metabolic activation.

	Salmonella Typhimurium Strain			
	TA-98	TA-98 NR	TA-98 1,8DNP$_6$	TA-98 NR/BR
Mutagenicity Revertants/m^3 in the mine air	1,382,088	234,436	4711	1570

* This data was taken from reference 10.

PAHs and NO_2 are known components of diesel exhaust (2,7) ii) NO_2 has been shown to react with PAHs even at low temperatures (8) and iii) the nitroPAHs are amoung the most highly mutagenic compounds known (2,9).

The following attempts have been made to determine the actual nitroPAHs that were responsible for the high mutagenicity of the Dieseler III exhaust extract. Although the mutagenicity of the diesel exhaust extract was very high in every sample collected in the underground environment when the Dieseler III catalytic converter was used (1), and although the samples were collected on several different occasions in different underground environments, the underground results could not be duplicated in the laboratory using dilution tunnels, Table 2 (10). In fact, the mutagenicity of only one out of 10 samples collected in the laboratory was even in the range that had been found in the underground environment. Even in the sample with the elevated mutagenicity, the mutagenicity was at the low end of the range found in underground environments, i.e., the highest mutagenicity was only 423,373 revertants/m^3 in the laboratory test whereas the mutagenicities of the Deiesler III exhaust extracts from the underground air samples ranged from 371,688 to 1,825,250 revertants/m^3 (10).

Table 2. The mutagenicity of diesel exhaust extracts from filters collected in a dilution tunnel at a dilution ratio of 16:1 when a Deutz F6L 714 diesel fitted with a Dieseler III catalytic converter was operated at an engine speed of 2200 rpm and 7/8 load.

Sample	Mutagenicity in revertants/m^3
1	30,388
2	11,924
3	423,373
4	114,096
5	14,231
6	19,365
7	73,399
8	22,984
9	9,925
10	65,338
Average	78,502

A second attempt to obtain highly mutagenic diesel exhaust extract from a Dieseler III catalytic converter involved collecting samples in an underground environment (11). In this experiment, high volume samples of diesel particulate were collected downstream from a Deutz diesel fitted with a Dieseler III catalytic converter. These samples, which should have duplicated the earlier underground samples which were highly mutagenic, were to be analyzed for nitroPAHs. The results would demonstrate which, if any, of the nitroPAHs were responsible for the high mutagenicity of the Dieseler III exhaust system.

Unfortunately, the results of this test were negative. In fact, the average mutagenicity of the mine air downstream from the diesel with the Dieseler III catalyst was normal, i.e., close to that found when no exhaust treatment devices were on the diesel. In fact, the average air collected downstream from the engine with the Dieseler III catalyst was slightly less mutagenic than the air downstream from the bare engine, i.e., the mutagenicity downstream from the engine with the Dieseler III catalyst was only 227 revertants/m^3 whereas the mutagenicity of the air collected downstream from the bare engine was 375 revertants/m^3 (11). The failure to collect highly mutagenic samples was attributed tentatively to the mine layout that was used in this test. In this experiment, the diesel mucked ore at the bottom of a ramp with a 20 % grade. Then it carried the ore up the ramp and dumped it onto the ore train. On the return

part of the LHD cycle, the diesel went down the ramp and began mucking.

The Dieseler III catalyst is believed to work by capturing the diesel particulate released by the engine when the engine is working at low power. Then, the PAHs on the trapped particulate are nitrated (converted into the nitroPAHs) when the hot nitrogen dioxide passes over the particulate trapped in the catalyst. Finally, the nitrated particulate is blown out of the catalyst into the mine air when the diesel is working at full power. Miners working around or downstream from diesels fitted with Dieseler III catalysts would be exposed to the highly mutagenic nitroPAHs.

In this experiment, the diesel was working at high power during the mucking and the upward (loaded) tramming portion of the LHD cycle. Therefore, the diesel particulate would only be stored during the dumping (approximatly 10 sec) and the short trip back down the ramp. Because the storage time was short, little or none of the PAHs on the particulate trapped in the catalyst during the dumping and downward tramming portions of the LHD cycle would have reacted with the NO_2 in the exhaust and have been nitrated (converted into nitroPAHs). As a result, the mutagenicity of the diesel exhaust extract was low, there were obviously too few nitroPAHs to analyze, and the experiment failed.

The work undertaken in this study involved collecting high volume samples of diesel particulate downstream from two different diesels fitted with Dieseler III catalysts. In this experiment, however, the sampling was done in a specific mine layout where there was a long level tramming portion in the LHD cycle. Particulate could be stored in the Diesler III catalyst and nitrated during the long tramming and dumping operation. Then, the highly mutagenic nitroPAHs in the catalyst would be blown out of the catalyst into the mine air when the diesel worked at maximum power during the mucking portion of the LHD cycle. This would hopefully eliminate the problem encountered in the previous study and provide samples of diesel exhaust that contained sufficient nitroPAHs to be identified.

RESULTS AND DISCUSSION

TEST SITE

The high volume diesel exhaust samples required for this study of the mutagens released from Diesler III catalytic converters were collected in Panel 142 on the 2375 minus 40 foot level of Falconbridge Limited's Strathcona Mine. The level plan for the sampling area is shown in Figure 2. The main ventilation of 36,000 cfm came from the dump site and past the entrance to Panel 142 where the sampling was done. A vent tube provided 5000 cfm of air from the tramway to the face of the muck pile in Panel 142. The return air carried the diesel particulate from the mucking operation past the high volume samplers, Figure 2.

SAMPLE COLLECTION

The LHD cycle used in this study was ideal for this experiment. The ore was mucked at a draw point which was approximately 90 feet inside Panel 142, Figure 2. The ore was mucked with the diesel operating on remote. Then, the diesel backed out of the Panel and transported the ore 750 feet on the 2375 minus 40 foot level to the dump site. After the ore was dumped, the scoop tram returned along the level to the mucking site where another load of ore was picked up. Since the tramming protion of the LHD cycle was

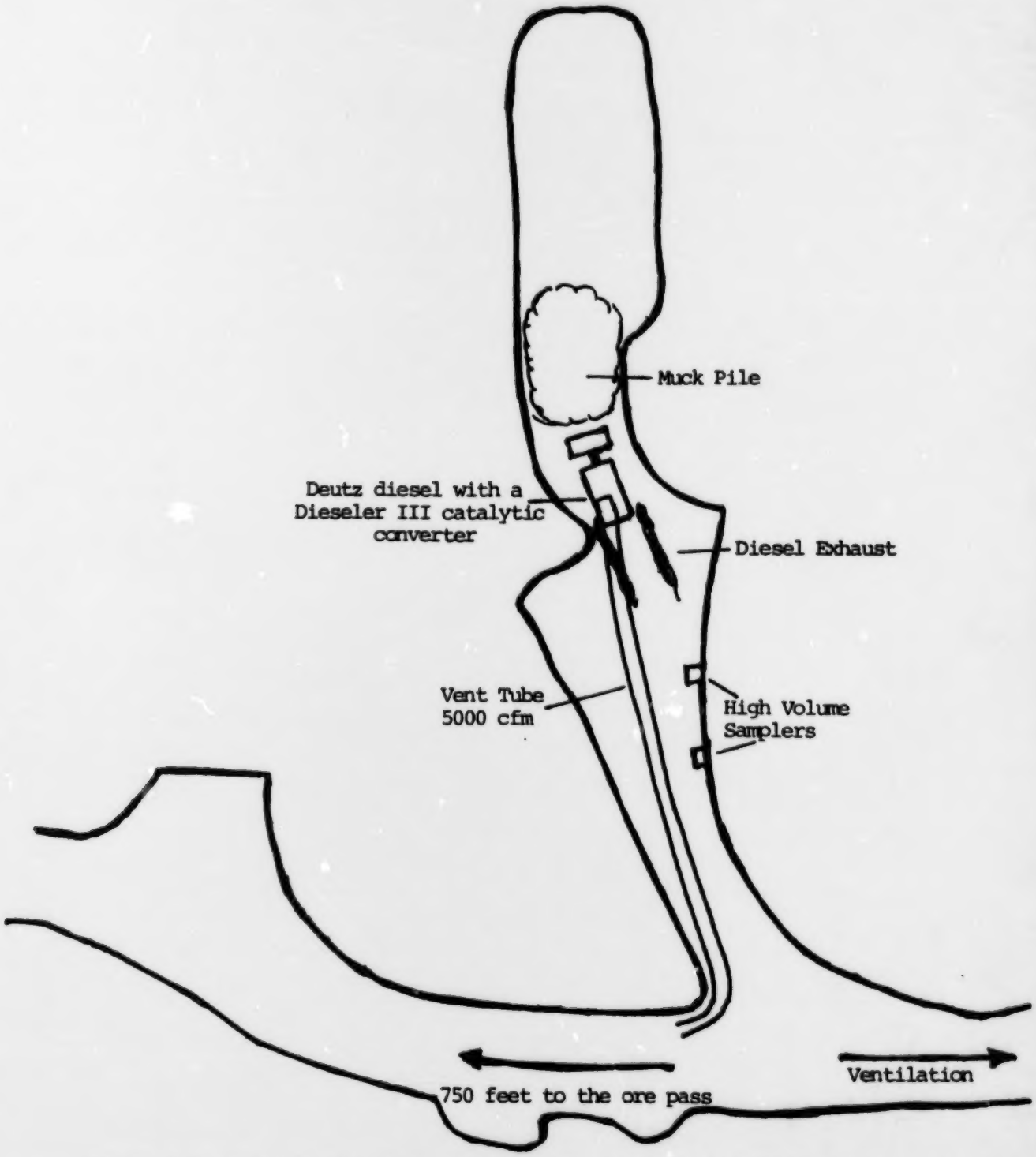

Figure 2. Sampling site in Panel 142 on the 2375 - 40 foot level of Falconbridge Limited's Strathcona mine.

done on a level portion of the mine, there was ample opportunity for the diesel particulate to be stored in the catalyst and nitrated.

In the average LHD cycle used throughout the sample collection, the diesel ran at idle for 1 minute. This portion of the LHD cycle occurred in front of, i.e., between the muck pile and the high volume samplers. This idle was required because the mucking was done while the diesel was operated on remote control. The operator took approximataly 30 seconds to change from manual to remote when the diesel entered and left the sampling area. On average, the mucking portion of the LHD cycle took 1.5 minutes and the tramming and dumping lasted 4 minutes.

Two simultaneous (one set of) high volume samples of diesel particulate were collected downstream from two different Deutz diesels fitted with Dieseler III cataytic converters. One of the diesels, (# 53) was a 174 horsepower F8L-714 while the second diesel (# 413) was a 145 horsepower F6L-714. The high volume samplers were situated at the side of the drift approximately 50 and 60 feet downstream from the exhaust ports of the diesel when it was mucking. The two samplers, labelled front (closest to the muck pile) and back, were 10 feet apart, approximately 6 feet above the floor of the drift. This was done so any dust raised as the diesel passed the sampling site was not collected on the high volume filters. Finally, all of the samples were

collected until the flow rate through the high volume filter had been reduced to 80 % of its original flow rate. Then, the samples were removed and stored in the dark in the freezer until they could be extracted for the Ames and nitroPAH analyses.

Drager tubes were used to determine the carbon dioxide concentration at the sampling site at various times during both the mucking and tramming portions of the LHD cycle. The average carbon dioxide levels during the mucking and the tramming are given in Table 3 along with the details of the sample collection. Drager tube sampling indicated that the nitrogen dioxide levels remained below 1 ppm at the sampling site throughout the experiment.

Six sets of diesel exhaust samples were collected downstream from the diesels, Table 3. The first two sets of samples (A and B) were collected continuously, i.e., the high volume samplers were run even when the diesel was not in the panel mucking ore.

The four other sets of samples, C - F, were collected only when the diesel was mucking ore. This was done in an attempt to only collect the exhaust from the mucking portion of the LHD cycle, i.e., when the diesel should be expelling stored diesel particulate that should be rich in nitroPAHs. This sampling technique should maximize the amount of nitrated PAHs in the sample and provide an extract with the highest possible mutagenicity because the samples were not

Table 3. The sampling conditions for the samples collected downstream from Deutz 714 series diesels fitted with Dieseler III catalytic converters

Diesel Number	Catalyst	Sample Number	Sampling Time (min.)	Volume of Air (m^3)	Average* Percent CO_2	Sampling Method
53	CANMET	A Front	38.0	46.81		Total time including tramming
53	CANMET	A Back	31.0	37.75		
		A Combined		42.28	N.M.	
53	CANMET	B Front	70.0	78.15		Total time including tramming
53	CANMET	B Back	73.0	85.38		
		B Combined		81.77	0.18 (0.08)	
53	CANMET	C Front	58.0	66.83		For 35 sec after diesel left sampling site
53	CANMET	C Back	59.75	71.27		
		C Combined		69.05	0.18 (0.12)	
413	Falco	D Front	31.77	36.44		For 1 min after diesel left sampling site
413	Falco	D Back	43.0	49.64		
		D Combined		43.04	0.18	

Table 3 Cont. The sampling conditions for the samples collected downstream from Deutz 714 series diesels fitted with Dieseler III catalytic converters

Diesel Number	Catalyst	Sample Number	Sampling Time (min.)	Volume of Air (m^3)	Average* Percent CO_2	Sampling Method
53	CANMET	E Front	30.25	34.38		For 1 min after diesel left sampling site
53	CANMET	E Back	30.25	36.36		
		E Combined		35.37	0.21	
413	Falco	F Front	25.67	27.77		For 1 min after diesel left sampling site
413	Falco	F Back	25.67	29.82		
		F Combined		28.80	0.19 (0.15)	

* The values are the average percent CO_2 found during mucking. The values in brackets are the percent CO_2 found during tramming, i.e., when the diesel was not in the drift.

diluted with exhaust picked up by the 5000 cfm vent tube from the tramming portion of the LHD cycle, see Figure 2. Sample set C was collected by running the high volume samplers from the time the diesel reached the muck pile, throughout the mucking and for 35 seconds after the diesel passed beyond the high volume samplers on the way out of the drift. For the sample sets C-F, the high volume samplers were run for 1 minute after the diesel had passed the samplers on the way out of the drift. The time was increased because the hot exhaust from the engine could be felt in front of the samplers for approximately 1 minute after the diesel had left the mucking site. It was assumed that the high temperature of the air was caused by the diesel exhaust gases and particulate that had not been swept out of the sampling area. As a result, the samples were collected until the drop in temperature signified that most, if not all, of the diesel particulate released in the mucking portion of the study had been swept from the sampling area.

AMES BIOASSAYS

Each set of high volume diesel exhaust samples collected in the underground environment were combined to provide two identical samples. One of each set of identical samples was sent for the Ames bioassay with Salmonella Typhimurium tester strain TA-98 without metabolic activation. The second identical sample was set aside in the freezer so it

could be analyzed for nitroPAHs. The two identical samples were generated by cutting each high volume sample (the front and the back high volume filters) into two equal pieces. Then one piece of each filter, i.e., half of the front filter and half of the back filter from each set were combined into one sample. The other two halves of these filters were combined to form the other identical sample for the nitroPAH assay.

The results of the Ames bioassays are shown in Table 4. The mutagenicity of these samples were much lower than expected. In fact, no mutagens were detected in five of the six samples collected downstream from the diesels fitted with Dieseler III catalytic converter. In the one sample which was mutagenic, the mutagenicity was only 342 revertants/m^3 in the mine air. This value is, in fact, in the range usually found for the bare engine. The normal response found in this sample suggests that the other samples contained too little diesel exhaust to show a positive result in the Ames bioassay. The unusually low mutagenicity was not expected because the weight of the organic extracts were normal, i.e., the extract weights, which ranged from 13 to 27 mg, often range between 20 and 30 mg. (1). Also, the volume of air sampled was not unusually low, i.e., although it was on the low end of the normal volume of air collected, the samples were only collected during the mucking portion of the LHD cycle and should

Table 4. The mutagenicities* of the high volume filter extracts of diesel exhaust samples collected downstream from Deutz 714 series diesels fitted with Dieseler III catalytic converters in Panel 142 on the 2375 minus 40 foot level of Falconbridge Ltd's Strathcona mine.

Sample	Scoop Tram	Dieseler III catalyst	Weight of soluble organic extract (mg)	Specific activity (Rev./µg)	Volume of air sampled (m^3)	Revertants m^3 in the mine air
A	53	CANMET	16.4	Negative	42.28	-
B	53	CANMET	20.1	Negative	81.77	-
C	53	CANMET	27.0	Negative	69.05	-
D	413	FALCO	22.2	Negative	43.04	-
E	53	CANMET	24.7	0.49	35.37	342
F	413	FALCO	13.0	Negative	28.80	-

* The details of the Ames analyses are presented in Appendix I.

have been rich in the diesel exhaust (nitroPAHs) released during mucking.

The important observation, however, is that the mutagenicity of the extracts from two different Dieseler III catalytic converter samples was low. If even small amounts of nitroPAHs had been present in these samples, a high mutagenicity would have been found. For example, the nitropyrenes should be present in highest concentration because pyrene is present in the highest concentration in the mine air (1,2). The average specific mutagenicity of the three most likely dinitropyrene isomers is 666 revertants/ng (2). Thus, even if the concentration of dinitropyrenes at the sampling site were at the extremely low level of 1 ng/m^3, the specific mutagenicity would have been at least 1.3 and could have been as high as 2.7 revertants/µg. In fact, the average specific mutagenicities of Dieseler III samples previously collected from different engines in the underground environment was 72 revertants/µg indicating that significant quantities of nitroPAHs were present in the samples. In any case, since the expected values are more than five times higher than the highest value found in this study, it is clear that the nitroPAH concentrations in the samples are very low. As a result, the samples were not sent for a nitroPAH analysis.

The samples collected from the Dieseler III catalytic converter were different in two ways from the usual diesel

exhaust extracts. First, the samples were more toxic to the Salmonella tester strain TA-98 than any other samples of diesel exhaust from either a bare engine or from diesels fitted with a wide variety of catalysts and filters in over eight years of testing (12). For example, toxicity to the TA-98 Salmonella bacteria were found in samples containing as little as 150 µg of the diesel exhaust extract. Normally, toxicity is not found until ≥ 1000 µg of extract are used in the Ames bioassy (1). The compound(s) responsible for this toxic response have not been identified. As a result, the health implications arising from the presence of these compound(s) are also not known.

The second difference between these Dieseler III and other diesel exhaust extract samples is that a significant amount of the soluble organic fraction (the material extracted from the high volumer filter with dichloromethane, the solvent used to extract the mutagens, the PAHs, and their derivatives from the high volume filters) did not dissolve in the DMSO that is used to prepare the solution that is spotted on the plates containing the Salmonella bacteria used in the Ames analysis. The material that did not dissolve in the DMSO has not beeen identified and we do not know whether it arose from the Dieseler III catalyst or from the mine.

C O N C L.U S I C N

An ideal sampling location with a long (more than 800 feet) level tramming distance from the sampling site to the ore pass, was used for this study. Thus, the PAHs and diesel particulate should have been stored and nitrated (converted into nitroPAHs) in the Dieseler III catalytic converter during the tramming and dumping portions of the LHD cycle. As a result, the high volume samples collected downstream from the mucking site should have contained significant amounts of nitroPAHs and been highly mutagenic. In spite of this, the soluble organic extracts from the high volume samples of diesel exhaust collected downstream from two different Deutz 714 series diesels fitted with Dieseler III catalytic converters contained little or no mutagenic material. In fact, only one out of six samples showed any mutagenicity and that sample was only as mutagenic as the organic extracts from high volume samples collected downstream from bare engines. Thus, the exhaust from the Dieseler III catalysts used in this study did not contain high concentrations of the nitroPAHs.

The organic extracts of the high volume samples collected in this study differed from those found in our previous work in two ways. First, they were unusual because they contained significant amounts of material that did not dissolve in the DMSO solution that was spotted on the

Salmonella bacteria plates in the Ames bioassays. Normally, the whole extract dissolves the the DMSO. The second difference from the normal extracts is that these extracts were much more toxic to the Salmonella bacteria used in the Ames bioassays. Neither the DMSO insoluble material nor the cause of the toxicity has been identified.

A C K N O W L E G M E N T S

We are indebted to Falconbridge Limited for allowing us access to their Strathcona mine. Without thier cooperation, this work could not have been undertaken. Particular thanks goes to Ms. Heather Walker and to Mr. Gary Lampe who organized the sampling. The assistance of Mr. Stan Bouffard during the sampling is also appreciated.

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A P P E N D I X I

November 10, 1988
88-48-30011-011

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Dear Ken:

Enclosed are the single strain (TA-98) mutagenicity test results for the six samples submitted. The soluble organic fraction for each sample was:

Lab#	Sample Identification		SOF
A	Falco June 20 #1	1/2 front, 1/2 back	16.4 mg
B	June 20 #2	1/2 front, 1/2 back	20.1 mg
C	June 21	1/2 front, 1/2 back	27.0 mg
D	August 8 Scoop	1/2 front, 1/2 back	22.2 mg
E	Sample F2 August 8 Scoop 143	1/2 front, 1/2 back	13.0 mg
F	Sample E2 August 8 Scoop 53	1/2 front, 1/2 back	24.7 mg

MUTAGENICITY TEST RESULTS (TA-98 without Activation)

The positive and negative assay controls conducted with both sets of samples indicate the assay system was functioning properly.

For a sample to be classified as positive and mutagenic it must show a response of 2 1/2 times the negative control. The sample should also exhibit a dose-response.

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Samples A to E are negative and non-mutagenic. Sample F is weakly mutagenic with a dose-response between 50 and 150 µg/plate. This sample has a specific activity of 0.49 revertants/µg. All samples were tested to a toxic concentration ensuring that a positive response was not missed because of insufficient material.

The samples submitted are highly toxic to the Salmonella tester strain. They contain a material that was soluble in dichloromethane and insoluble in DMSO. This unknown material made up what appeared to be a large part of the SOF and the samples were not characteristic of diesel particulate. I suspect the samples were composed mostly of non-diesel particulates.

Please call if you have any questions regarding these results.

A. J. Horton

A. J. Horton
Mutagenic Services
Energy & Environmental
Engineering Division

AJH/vp

Mutagenicity Results for Salmonella
Tester Strain TA-98 without Metabolic Activation

Concentration µg/Plate	Histidine Revertants			Mean	Comments
Sample A (Falco June 20 #1)					
1000	Toxic			0	Toxic
400	41	46	41	43	Slight Toxicity
300	44	42	46	44	Slight Toxicity
200	37	32	39	36	
150	52	32	49	46	
100	52	43	42	46	
50	29	31	33	31	
10	16	31	26	24	
Sample B (June 20 #2)					
1000	Toxic			0	Toxic
400	45	39	43	42	Slight Toxicity
300	38	25	19	27	Slight Toxicity
200	42	23	48	38	Slight Toxicity
150	39	44	42	42	
100	34	23	39	32	
50	25	34	27	29	
10	24	20	23	22	
Sample C (June 21)					
1000	Toxic			0	Toxic
400	44	44	36	41	Slight Toxicity
300	34	35	32	34	
200	26	35	28	30	
150	51	56	38	48	
100	51	57	36	48	
50	35	30	23	29	
10	18	25	27	23	
Sample D (August 8 Scoop)					
1000	Toxic			0	Toxic
400	32	27	30	30	Slight Toxicity
300	20	22	29	24	
200	13	17	21	17	
150	24	21	23	23	
100	72	65	50	62	
50	28	47	41	39	
10	31	32	22	28	

Concentration µg/Plate	Histidine Revertants	Mean	Comments
Negative Control			
A to D	29 28 33 24 39	31	OK
Positive Control			
A to D	442 416 494	451	OK
Sample E (Sample F2 August 8 Scoop 143)			
1000	Toxic	0	No Lawn
400	Toxic	0	No Lawn
300	Toxic	0	No Lawn
200	Toxic	0	No Lawn
150	Toxic	0	No Lawn
100	39 19 Tox	?	Toxicity
50	87 102 84	91	
10	23 24 31	26	
Sample F (Sample E2 August 8 Scoop 53)			
1000	Toxic	0	No Lawn
400	Toxic	0	No Lawn
300	Toxic	0	No Lawn
200	Toxic	0	No Lawn
150	93 84 93	90	
100	71 58 77	71	
50	38 51 37	42	
10	27 28 14	23	
Negative Control			
E to F	22 34 23 26 27	26	OK
Positive Control			
E to F	443 525 443	470	OK